Arch Jpn Chir 52(6), 841~853, Nov., 1983

臨　　床

Cancer Immunotherapy by Local Transfer of Autologous T Lymplocytes Cultured with T Cell Growth Factor and Autologous Tumor Extract: A Clinical Trial

NORIMICHI KAN, KAZUHISA OHGAKI, NOBUYASU YAMASAKI, TAISUKE HORI, NOBORU NAKAYAMA, YOSHINORI NIO, TAKASHI INAMOTO and YORINORI HIKASA

The Second Department of Surgery, Faculty of Medicine, Kyoto University
(Director: Prof. Dr. YORINORI HIKASA)
Received for Publication, July 10, 1983.

Abstract

Cytotoxicity and proliferation of lymphocytes from cancer patients were enhanced by in vitro culture with autologous tumor extract and T cell growth factor (TCGF). These cultured lymphocytes were locally re-infused to the respective patients. Twenty-five patients received this adoptive immunotherapy. The total number of infused cells ranged from 1×10^7 to 5×10^9. Thirteen of these patients had resectable advanced cancers and received this immunotherapy in combination with surgical treatment. Twelve patients had recurrent or disseminated cencer with measurable lesions before the treatment. Clinical or histological improvements were observed in 7 of 12 patients. ^{51}Cr release test with autologous cancer cells was studied in 5 patients. Significant cytotoxic activity of cultured lymphocytes against autologous tumor cells was observed in 4 of them; cytolysis against allogeneic target (K562 cells) was detected in all cultured lymphocytes tested.

Introduction

Lymphocytes of cancer patients are suppressed by several humoral[6,7] or cellular[2,3] mechanisms and, as a result, they demonstrate only weak antitumor activity against autologous tumor cells. Adoptive transfer of autologous lymphocytes which were re-educated specifically in vitro was speculated to be benefical in cancer immunotherapy[12]. However, early attempts with this type of therapy had been unsuccessful for several reasons: (1) the cytotoxic activity of cultured

Key words: Adoptive immunotherapy, T cell growth factor, Soluble tumor antigen, Cytotoxic T lymphocyte, Mixed lymphocyto-tumor culture.
索引語：癌免疫療法，T細胞増殖因子，腫瘍抗原，リンパ球腫瘍混合培養，抗腫瘍Tリンパ球.
Present address: The Second Department of Surgery, Faculty of Medicinl, Kyoto University, 54 Kawara-cho, Shogoin, Sakyo-ku, Kyoto 606, Japan.

lymphocytes against autologous cancer cells was difficult to be induced; (2) proliferation of cytotoxic T cells had been impossible until the discovery of TCGF by GALLO et al;[15] (3) in vivo kinetics and the administration route of cultured cells had not been sufficiently clarified by experimental models.

Recently, it has been shown by several authors that lymphocytes of cancer patients could become cytotoxic against autologous cancer cells when they were cultured in TCGF[11,18]. In addition, we found that the cytotoxic activities of lymphocytes of tumor bearers could be augmented by the addition of autologous tumor extract in experimental studies[16]. Concerning the in vivo effect of lymphocytes cultured in TCGF, some authors[13] and we[8] clarified that local administration brought favourable therapeutic effects while intravenous administration brought little or no effect. On the basis of these experimental studies, we have attempted to transfer autologous lymphocytes, which had proliferated and had been re-educated in vitro with both ultrasonicated soluble extracts of autologou tumor tissues and TCGF, to the patients locally.

Patients and Methods

Patients

The twenty-five patients who received this therapy consisted of 15 breast cancers, 9 gastrointestinal cancers and one thyroid cancer (Table 1). Twelve patients had measurable tumors at the time they received this therapy. Thirteen patients had no measurable tumors because the therapy was done after the surgical resection of the tumors. Some of the patients received this immunotherapy in combination with chemotherapy.

Ultrasonicated tumor autigens

The tumor tissues were minced with surgical sharp scissors, suspended in 10 m*l*/cm³ of RPMI 1640 medium, and disrupted by ultrasonication (20 K Hz, 105 W for 10 minutes). After the centrifugation (15,000 G, 90 minutes) the supernatant was passed through a 0.22 μm millipore-filter and stored at −80°C until use. Protein concentration measured by Lowrye's method ranged from 5 mg/m*l* to 1 mg/m*l* when prepared from solid tumors. The ultrasonication was performed for 90 seconds for tumor cells obtained from carcinomatous ascites or pleural effusion.

Preparation and culture of lymphocytes

Peripheral heparinized blood of the patient was overlayered on the Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) and was centrifuged at 500 g for 40 minutes. Interface was collected and washed 3 times with the Hanks' balanced salt solution (HBSS) by centrifugation. In one patient (Patient 5) lymphocytes prepared from ascites were used as the source of cultured lymphocytes.

Lymphocytes of patients were cultured in RPMI 1640 supplemented with 20% TCGF, 10% heat-inactivated human AB serum, 5×10^{-5} M 2-Mercaptoethanol (2 ME), 20 mM Hepes, 0.2 μg/m*l* Fungison, 100 μg/m*l* of Gentamycin, and 1〜5 vol % of soluble antigen. First culture of lymphocytes (5×10^5 cells/m*l*) was performed for 5 days, and second or third culture (1×10^5/m*l*)

Table 1. The patients who received adoptive immunotherapy

a.			b.		c.	d.	e.	f.
A) With measurable lesion								
1	KA	76M	esophagus	(rec.)	PL. PE.	PE.	1	1.5D
2	NH	55F	breast	(rec.)	BO. LOC. PL.	PL. LOC.	5	0.1D
3	MM	68M	colon	(rec.)	LV. PE.	PE.	16	2D
4	SO	45F	breast	(prim. IV)	LOC. int. mammary a.		19	2D
5	KK	62F	rectum	(rec.)	LV. PE.	PE.	93	6D
6	KN	63F	thyroid	(rec.)	LOC.	LOC.	118	12A
7	CN	54F	stomach	(rec.)	PE.	mesenteric a.	50	2D
8	FB	53F	breast	(prim. IV)	LOC. BO int. mammary a.	LOC	560	10D
9	KM	62F	rectum	(rec.)	LV. PE.	hepatic a.	74	5D
10	NT	62F	stomach	(prim. IV)	PE.	PE.	24	8A
11	JU	35M	rectum	(rec.)	LV. PE. LG.	heaptic a.	110	2D
12	KK	51F	breast	(rec.)	LV.	hepatic a.	343	6D
B) Without measurable lesion (combined with surgery)								
13	HY	53F	breast	(prim III)		Op. field	12	14A
14	TK	59F	breast	(prim III)		Op. field	16	12A
15	CY	41F	breast	(prim III)		Op. field	7	12A
16	NH	57M	rectum	(prim III)		Op. field	34	10A
17	YT	73M	rectum	(prim II)		Op. field	16	10A
18	KK	45F	breast	(prim III)		Op. field	61	8A
19	KM	40F	breast	(prim III)		Op. field	85	7A
20	YA	49F	breast	(prim III)		subclavian a.	166	7A
21	KN	50F	breast	(prim III)		Op. field	96	6A
22	SY	49F	breast	(prim III)		Op. field	93	5A
23	KY	50F	breast	(prim III)		subclavian a.	71	4A
24	KY	50F	breast	(prim III)		Op. field	50	4A
25	MO	61F	breast	(prim III)		Op. field	52	4A

a) patient number, age and sex M (male) or F (female)
b) primary lesion, recurrent (rec.) or primary (prim.) case and TNM staging
c) lesion at the therapy, pleural cavity (PL) peritoneal cavity (PE) bone (BO) local or regional (LOC) liver (LV) lung (LG)
d) site of lymphocyte injection, peritoneal cavity (PE), pleural cavity (PL) intratumoral (LOC) into the artery (internal mammary a, mesenteric a, hepatic a, subclavian a.) into the operative field (Op. field)
e) the number of the cultured lymphocytes injected ($\times 10^7$ cells)
f) survival (months) after the initiation of the therapy, dead (D) or alive (A)

for 4 days. After the termination of culture, a part of the secondarily cultured lymphocytes and all of tertiarily cultured lymphocytes were used for this therapy. Cultures were repeated once or twice a week. Linbro 2-m*l* wells (Cat No. 76–033–05, Flow Laboratories, Virginia) or Nunclon 800-m*l* flasks (Cat. No. 1–56502, Nunclon, Denmark) were used for these cultures.

TCGF

Spleens obtained from the patients who underwent splenectomy for gastric cancer, hypersplenism, or aplastic anemia were minced and passed through a #100 stainless steel mesh. Mononuclear cells were harvested by the same method used for lymphocytes of peripheral blood. After washing three times, mononucleated cells (1×10^6 cells/m*l*) were suspended in RPMI 1640

containing 0.08% PHA-P (Difco, Detroit, Michigan), 5×10^{-5} M 2ME, 20 mM Hepes, 2% human AB serum and 100 μg/ml Gentamycin, and cultured for 3 days at 37°C with 5% CO_2 in air. The supernatant was collected by centrifugation and stored at −20°C. These supernatants were concentrated 5-fold using an Amicon membrane (YM-5) and passed through a 0.22 μm Millipore-filter before use. Spleens from patients over 70 years old or with Hepatitis B antigen were excluded.

Transfer of lymphocytes

These cultured lymphocytes were harvested and washed 3 times with HBSS, suspended in 5 to 50 ml HBSS, and injected via the following routes.

1) Intratumoral injection (for superficial solid tumors).

2) Intrapleural or intraperitoneal injection (for carcinomatous ascites or pleural effusion).

3) Intraarterial injection, (into the hepatic artery for metastatic liver tumors, into the internal mammary artery and/or the subclavian artery for breast cancers).

4) Injection into the operative field (into the supraclavicular area, parasternal area or subclavicular area after operation for breast cancers, presacral area for rectal cancers).

^{51}Cr release test

The solid tumor tissues were minced with sharp scissors and were treated with phosphate-buffered saline containing 2 U/ml collagenase (Worthington, Firehold, New Jersey) for 40 minutes and washed 3 times with HBSS. The tumor cells in carcinomatous ascites or pleural effusion were simply centrifuged, resuspended in HBSS, and subjected to discontinuous Ficoll-gradient centrifugation to eliminate lymphocytes and dead cells. Briefly, 100% of Ficoll-paque, 80% of Ficoll, and tumor cell suspension were consecutively overlayered, and centrifuged at 500 g for 40 minutes. The 80% Ficoll interface was collected as the tumor-rich layer. After 3 washings with HBSS, when necessary, tumor cells were frozen in a program-freezer in PRMI 1640 containing 20% human AB serum and 20% DMSO, and stored at −190°C until use.

The autologous tumor cells together with 100 μCi of $^{51}Cr/5 \times 10^6$ cell were incubated at 37°C for 40 minutes. After washing 3 times the target cells (2×10^4) were incubated in 0.2 ml of medium (RPMI 1640 supplemented with 10% human AB serum, 20 mM Hepes) mixed with 2×10^5 or 1×10^6 effector cells, in a Linbro round-bottomed microtest plate, for 8–10 hrs at 37°C with 5% CO_2 in air. After the incubation, supernatants were harvested with a Titertek supernatant collection system (Flow Laboratories). The radioactivity was measured with a gamma counter (Autowell Nucl. Chicago) % specific cytotoxicity was calculated as:

$$\% \text{ cytotoxicity} = \frac{\text{test release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}}.$$

Total release expresses lysis by 2% of Triton X and spontaneous release expresses lysis without effector cells.

In order to evaluate natural killer (NK) activities of the cultured lymphocytes, K562 cells serially maintained in our laboratory were used as the target.

Results

Proliferation of lymphocytes

Lymphocytes of the patients proliferated well with TCGF until the 13th day of culture, even when TCGF was prepared from the spleens of gastric cancer patients (Fig. 1). Further culture, however, produced only slight proliferation. Therefore, one unit of culture was terminated at day 13 and the proliferated lymphocytes were harvested. Repeated cultures using 20 m*l* of peripheral blood, once or twice a week, were performed for respective patients.

The total number of transferred lymphocytes was more than 10^9 in 5 patients and more than 10^8 in 22 patients.

Side effects

In patient 6, slight leucopenia was observed, but it was transient, and a hemogram showed a normal pattern.

In patient 12, after the transfer of 1×10^9 lymphocytes into the hepatic artery for liver metastasis, mild fever and dull hypochondrial pain occurred beginning 2 days after the injection and persisting for 2 to 3 days; whether this was due to tumor necrosis or not could not be determined.

Two days after the intrapleural transfer of lymphocytes one patient at the terminal stage (patient 2) died of respiratory failure; this death was not considered as a side effect of the therapy because she had already been in a cachexic state before the therapy.

Hepatitis occured in one case (patient 4), but it was probably due to the combined chemotherapeutic agents (Adriamycin and 5 Fluorouracil).

There was no other side effects such as high fever, or hypotension. Moreover, the patients who received this therapy in combination with surgical resection (patient 13–25) showed no side effects and all of them survived without signs of recurrence.

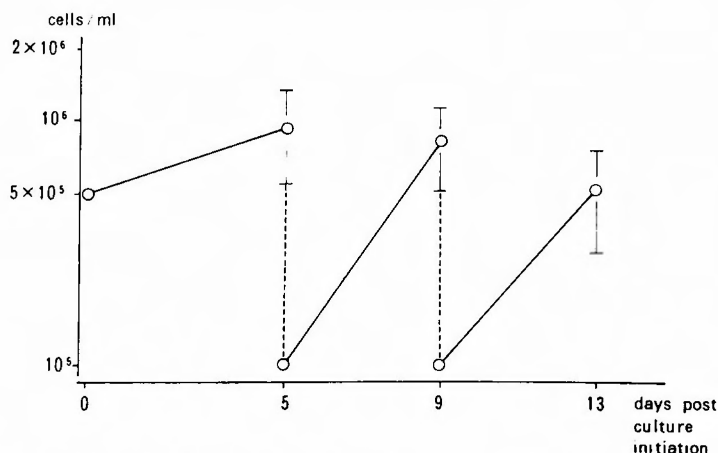

Fig. 1. Proliferation of lymphocytes cultured in TCGF. The number of lymphocytes per 1 m*l* of medium was counted using trypan-blue. The cell numbers were expressed as mean±SE in recent 30 cultures. Lymphocytes proliferated 85 fold (mean) at day 13 after culture initiation.

Table 2. ^{51}Cr. release cytotoxicity test using autologous cancer cells

Patient No.	Source of target cells	% Specific lysis			
		Pre-culture E/T		Post-culture E/T	
		50:1	10:1	50:1	10:1
2.	pleural effusion	1	0	26	8
3.	ascites	0	0	22	9
		(4)	(0)	(55)	(41)
5.	ascites	N.D.		25	8
				(57)	(40)
11.	ascites	0	0	52	17
		(2)	(0)	(62)	(40)
16.	tumor	0	0	0	0
		(7)	(3)	(59)	(49)

^{51}Cr release test was done as described in Materials & Methods.
Target cells: autologous tumor cells or ( ): K562 cells.

In vitro cytotoxicity test to autologous tumor cells

Four of 5 cases in which ^{51}Cr release test to autologous tumor cells was performed showed significant cytolysis (more than 20% of specific lysis with an E/T ratio of 50) (Table 2).

All of 4 cases tested showed remarkable cytolysis of allogeneic target cells (K562).

Clinical or histological effects

In 12 patients whose therapeutic effects were evaluable, namely, who had measurable lesion at the transferred site, 7 responded to the therapy. Five patients showed histological or cytological improvement, and 3 showed clinical improvement (Table 3). Although, these effects were observed only locally. No regression was observed at any area where the lymphocytes were not

Table 3. The patients who showed clinically or histologically favorable effects.

Patient No.	Combined therapy	Effect
1)	MMC 10 mg i.p. 10 days prior to therapy	cytology: class V before the therapy and class II after the therapy
4)	5FU and Adriamycin into the internal mammarian artery	no axillary tumor or subclavian tumor (which existed at the operation) at the autopsy
5)	no	tumor cell count/lymphocyte count from all ascites punctured before the therapy $4.9\times10^7/3.9\times10^7$, after the therapy $1.5\times10^7/6.5\times10^7$
8)	no	necrosis and lymphoid infiltration at the site of cultured lymphocytes injection
9)	MMC 20 mg and 5FU 1 g into the hepatic artery 20 days prior to therapy	CEA (by C.I.S. Kit) before therapy 49 ng/ml→after therapy 14 ng/ml (normal: < 10 ng/ml) tumor regression by computed tomography

injected.

Two cases in which both clinical and histological effects were observed will be presented.

Case Reports

Patient 3

A 68-year-old male underwent right-hemicolectomy for colon cancer with multiple liver metastasis 2 years before entry, after receiving infusion chemotherapy into the hepatic artery for liver metastasis, he was discharged. On August 8, 1981, he was admitted for abdominal distension due to carcinomatous ascites. Chemotherapy with oral administration of 5-Fluorouracil was ineffective and abdominal puncture was repeated. On October 17, 1981, and on November 2, 1981, cultured lymphocytes (2×10^7 and 14×10^7, respectively) were injected intraperitoneally. Abdominal distension decreased and further abdominal puncture was unnecessary. A transient decrease of CEA (by CEA Ire Solin Kit) was observed 10 days after the second injection (Fig. 2). After this therapy cytologic improvement from class V to class II of carcinomatous ascites was noted (Fig. 3). However, the patient had flapping tremor before the therapy and died of hepatic coma 2 months after the first injection.

Patient 6

A 63-year-old female underwent total thyroidectomy and bilateral radical neck dissection for

Fig. 2. Clinical course of patient 3.
a. The volume of malignant ascites punctured. (m*l*)
b. circumference of the abdomen. (cm)
c. cytological examination of the ascites (class)
d. CEA titer (ng/m*l*)

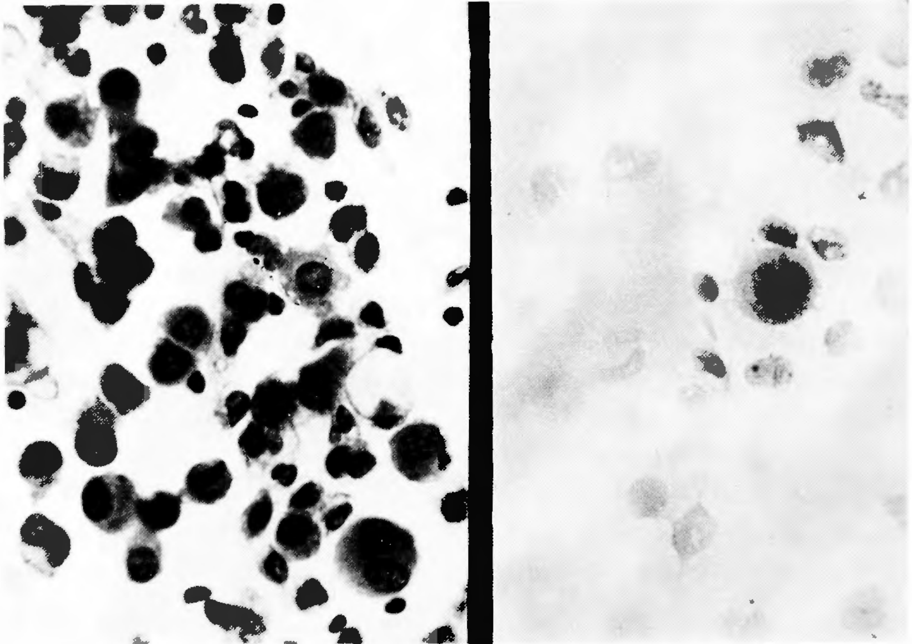

Fig. 3. Papanicolaou's stain of ascites (Patient 3)
Before the therapy (left) and after the therapy (right)
Microphotograph illustrating cytologic improvement from class V to class II (×400)

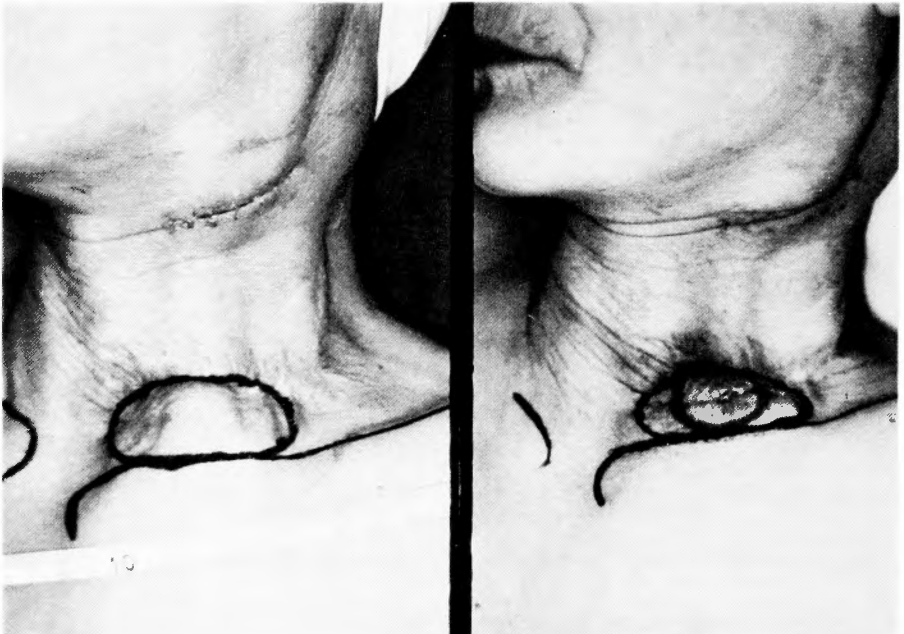

Fig. 4. Tumor regression after the immunotherapy (Patient 6)
Tumor diameter was 5 cm before the therapy and 3 cm after the therapy.

Fig. 5. Photograph of resected specimen (Patient 6) (× 1, HE stain)
Intact tumor tissue remained only in a small portion at the deep layer of the specimen. Other portion of the tumor shows various kind of histological degeneration (necrosis or fibrosis combinated with infiltration of lymphocytes).

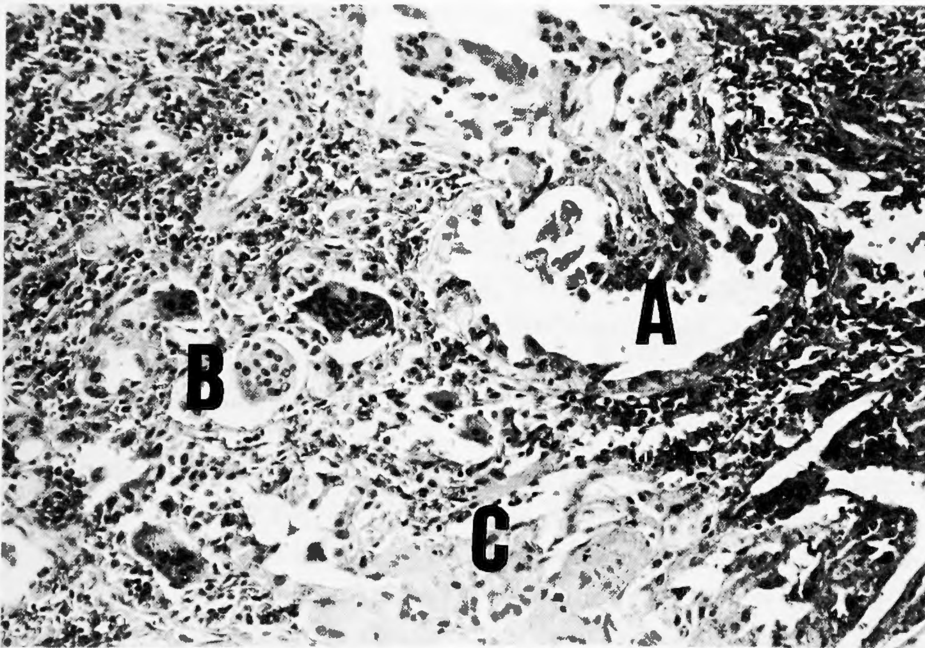

Fig. 6. Photomicrograph of higher power view (Patient 6, HE stain, ×200).
Various kinds of histological changes are illustrated.
A) intact papillary carcinoma
B) appearance of giant cells
C) vacuolar degeneration of carcinoma nest with marked lymphoid cell infiltration.

advanced thyroid cancer (papillary carcinoma) 7 years prior to entry and a recurrent tumor in left supraclavicular region was resected 5 years later. Recurrence was again observed in the same region and submandibular area in August 1981. On December 25, 1981, the submandibular nodule was resected and tumor extract was prepared. At the beginning of this therapy on January 5, 1982, a small biopsied specimen from the surface of the tumor revealed papillary carcinoma.

On day 20 after the beginning of the therapy, resection of the supraclavicular tumor was performed, at that time the tumor had regressed from 5 cm to 3 cm in diameter and had become movable after the intratumoral injection of 8×10^8 lymphocytes (Fig. 4, 5, 6). Tumor invasion to the subclavian vein was noted and the operation resulted in palliative one. Thus, an additional injection of 3×10^8 lymphocytes were performed into the operative field, near the vein. One year after the operation, the patient showed no signs of recurrence.

Discussion

In adoptive immunotherapy, the patients' own lymphocytes should be used because the transfer of allogeneic lymphocytes may cause the Graft-versus-Host reaction even with HLA compatible lymphocytes.[22]

In 1970, MOORE et al.[14] reported on their attempt to bring about proliferation of patients' lymphocytes and to transfer these cells back to the patients. As they used "Herpes-like leukovirus" to induce lymphocyte proliferation, the lymphocytes would be B cells and could not be expected to have anti-tumor activity. In addition, this approach was complicated by the possibility of viral infection. They reported chills and fever after the injection in almost all patients. In 1972, CHEEMA et al.[1] reported that the local transfer of antologous lymphocytes, which had been activated nonspecifically by PHA, had brought regression of 27 out of 29 nodules. But there was no theoretical evidence for this effect.

VOSE et al.[20] demonstrated that lymphocytes cultured with autologous tumor cells had anti-tumor activity against autologous tumor cells in sarcoma patients. But the clinical application of the adoptive immunotherapy using autologous lymphocytes has been difficult because of a decrease in the number of lymphocytes during culture. The recent discovery of TCGF by GALLO et al.[15] has enabled the enhancement of T cell proliferation while preserving the cells helper[21] or killer[4,5] activities, thus making it possible to use autologous lymphocytes in clinical application of adoptive immunotherapy.

ROSENBERG et al.[11] and VANKY et al.[18] reported that lymphocytes of sarcoma patients could easily acquire anti-tumor cytotoxicity by in vitro culture in TCGF. In our study, anti-cancer cytotoxicity of the lymphocytes could also be induced in 4 of 5 patients. Target cells in the remaining one case were prepared from a solid tumor by mincing and collagenase digestion. It is suspected that the antigenic determinant of tumor cells might have been shed off by enzymes[19] (Table 2). It is theoretically possible that the culturing in TCGF resulted in proliferation of suppressor T cells, especially in patients with terminal cancer. Although, the crude TCGF which we used contains many kind of lymphokines or other factors such as Interleukin-1,

Interleukin-2, γ-interferon and/or killer-helper factor[17], these factors other than Interleukin-2 (pure TCGF) might suppress the proliferation of suppressor cells.

In our culture system, ultrasonicated soluble tumor extracts were used as an antigen source in the mixed lymphocyte-tumor culture (MLTC). It is based on our experimental study[16] that repeated MLTC using soluble tumor extract and TCGF could induce high cytotoxic activity when lymphocytes of tumor-bearing animals were used as the source of effector cells. This cytotoxicity consists of the additive effect of tumor-specific activity induced by repeated MLTC and non-specific one obtained by the TCGF culture[9]. By the use of this ultrasonicated tumor antigen, in vitro re-education of lymphocytes from cancer patients with solid tumor such as breast cancer or gastrointestinal cancer, in which preparation of live tumor cells is difficult, has become possible and this type of cancer immunotherapy has become to be applicated clinically.

The administration route of cytotoxic lymphocytes cultured in TCGF is of most importance. In immunotherapy models[8,13], cytotoxic lymphocytes cultured in TCGF are effective only when they are transferred locally. Systemic administration resulted in failure in clinical trial of LOTZE et al.[10] and in experimental studies of MILLIS[13] and ours[8]. This may be due to differences in in vivo kinetics between cultured lymphocytes and fresh lymphocytes.

In our experimental models using mice, the cytotoxic effects of cultured lymphocytes were preserved well for at least 8 hours after intraperitoneal administration (unpublished data). The mice tolerated well the transfer of cultured, cytotoxic lymphocytes (2×10^8 cells per mouse) and survived for a long period without any complication. Our clinical trial was based on these findings.

The local transfer of cultured lymphocytes brought histological or clinical improvement in some terminal cancer patients. However, these effects were only partial and no remarkable life-prolonging effects could be obtained in patients with disseminated cancer probably because 1) the effector to target (E/T) ratio was too small, that is, the number of administered lymphocytes was too small to overcome the cancer mass: 2) there was the limitation of local therapy since terminal cancer patients usually have multiple lesion, and local administration of lymphocytes can treat only a part of them; 3) transferred lymphocytes could not counteract the suppressor factor or suppressor cells of the host.

However, we did observe the local effects even in the patients with disseminated disease. As patient 6 received no other therapy and patient 3 received only oral 5 FU, which had brought no effect before the immunotherapy, this therapy may bring about the favorable life-prolonging effects for the residual foci of locally advanced cancer which can not be treated completely by surgery, chemotherapy, or irradiation alone.

Acknoweldgment

A part of this work was done in collaboration with the Radioisotope Research Center, Kyoto University and we are grateful to Dr. KURIHARA and the other staff of the center· The authors also thank Dr. H. KODAMA for clinical support, Ms. TANAKA for technical assistance.

References

1) Cheema AR, Hersh EM: Local tumor immunotherapy with in vitro activated autochthonous lymphocytes. Cancer **29**: 982-987, 1972.
2) Eggers AE, Wunderlich JR: Suppressor cells in tumor-bearing mice capable of nonspecific blocking of in vitro immunization against transplant antigens. J Immunol **114**: 1554-1556, 1975.
3) Fujimoto S, Green MI, et al: Regulation of the immune response to tumor antigens I: Immunosuppressor cells in tumor bearing hosts. J Immunol **115**: 791-799, 1976.
4) Gillis S, Baker PE, et al: Long-term culture of human antigen-specific cytotoxic T cell lines. J Exp Med **148**: 1993-1998, 1978.
5) Gillis S, Smith KA: Long-term culture of tumour-specific cytotoxic T cells. Nature **268**: 154-156, 1977.
6) Hellström KE, Hellström I: Lymphocyte mediated cytotoxicity and blocking serum activity to antigens. Adv Immunol 209-277, 1974.
7) Kaliss N: Immunological enhancement of tumor homografts in mice. Cancer Res. **18**: 992-1003, 1958.
8) Kan N, Ohgaki K, et al: Specific adoptive immunotherapy by local transfer of cultured syngeneic spleen cells for mammary carcinoma in mice. Arch Jpn Chir **52**: 185-195, 1983.
9) Kedar E, Ikejiri BL, et al: Natural cell-mediated cytotoxicity in vitro and inhibition of tumor growth in vivo by murine lymphoid cells cultured with T cell growth factor (TCGF). Cancer Immunol Immunother **13**: 14-23, 1982.
10) Lotze MT, Line BR, et al: The in vivo distribution of autologous human and murine lymphoid cells grown in T cell sgrowth factor (TCGF): Implications for the adoptive immunotherapy of tumors. J Immunol **125**: 1487-1493, 1980.
11) Mazumder A, Grimm EA, et al: Lysis of fresh human solid tumors by autologous lymphocytes activated in vitro with lectins. Cancer Res **42**: 913-918, 1982.
12) McKahann CF: Approaches to immunotherapy. Cancer **34**: 1521-1531, 1974.
13) Millis GB, Carlson G, et al: Generation of cytotoxic lymphocytes to syngeneic tumors by using co-stimulator (Interleukin-2): In vivo activity. J Immunol 1980; **125**: 1904-1909, 1980.
14) Moore GE, Gerner RE: Cancer immunity-Hypothesis and clinical trial of lymphocytotherapy for malignant diseases. Ann Surg **172**: 733-739, 1970.
15) Morgan DA, Ruscetti FW, et al: Selective in vitro growth of T lymphocytes from normal human bone marrows. Science **193**: 1007-1008, 1976.
16) Nakayama N, Kan N, et al: Anti-tumor activity of spleen cells—its induction by MLTC using soluble antigen and TCGF. Proc Jpn Cancer Assoc **14**: 122 (Abstr.), 1982.
17) Simon PL, Farrar JJ, et al: Biochemical relationship between murine immune interferon and a killer helper factor. J Immunol **122**: 127-132, 1979.
18) Vanky F, Gorsky T, et al: Lysis of tumor biopsy cells by autologous T lymphocytes activated in mixed cultures and propagated with T cell growth factor. J Exp Med **155**: 83-95, 1982.
19) Vanky F, Klein E, et al: Human tumor-lymphocyte interaction in vitro. Cancer Immunol Immunother **5**: 63-69, 1978.
20) Vose BM, Vanky F, et al: IN VITRO generatino of cytotoxicity against autologous human tumour biopsy cells. Int J Cancer **21**: 588-593, 1978.
21) Watson J: Continuous proliferation of murine antigen-specific helper T lymphocytes in culture. J Exp Med **150**: 1510-1519, 1979.
22) Yonemoto RH, Terasaki PI: Cancer immunotherapy with HLA-compatible thoracic duct lymphocyte transplantation—a preliminary report. Cancer **30**: 1438-1443, 1972.

和文抄録

T細胞増殖因子（TCGF）及び可溶性自家腫瘍抗原を用いて培養した自己T細胞局所移入による癌治療の臨床経験

京都大学医学部外科学教室第2講座（主任：日笠頼則教授）
菅　典道，大垣　和久，山崎　信保，堀　泰祐，仁尾　義則
中山　昇，稲本　俊，日笠　頼則

癌患者のリンパ球を，自家腫瘍抗原及びT細胞増殖因子（TCGF）にて培養することにより，腫瘍障害性を与え，かつ増殖させ，各患者の病巣に局所注入した．25例の患者にこの免疫療法（adoptive immunotherapy）を行ない，注入しえたリンパ球数は1×10^7ケから5×10^9ケに及んだ．25例中13例は切除可能進行癌の術後補助療法として用い，残り12例は再発，末期癌中心で治療時に計測可能な病巣を有していた．臨床的或は組織学的な他覚的局所効果を後者12例中7例に認め得た．培養リンパ球の自己腫瘍障害性を^{51}Cr細胞障害試験にて検し得た5例中4例に有意の抗腫瘍活性を認め，更に検し得た全例に高度のNK活性（K562細胞障害性）を認めた．